Characterization and Oxidative Stability of Cold-pressed Sesame Oil Microcapsules Prepared by Complex Coacervation

Hui-Hui Dai, Xiao-Dong Li, An-Chi Wei*, Xue-De Wang, and Dong-Ying Wang

College of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, CHINA

Abstract: Although cold-pressed sesame oil (CPSO) possesses high nutritional value, its application in the food industry is limited due to its poor oxidative stability. The aim of this study was to enhance the oxidative stability of CPSO by complex coacervation microcapsule technology with gelatin and gum Arabic as wall materials. The characterization of CPSO microcapsules were evaluated by a particle image analyzer, a laser particle size distribution analyzer, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA). The encapsulation efficiency (EE) reached 90.25%. The average particle size of the microcapsules was approximately 117.1 μm and many oil droplets were encapsulated by complex coacervation to form a multinuclear spherical microcapsule. The FTIR study confirmed that the process of complex coacervation was formed between gelatin and gum Arabic by electrostatic interactions. The TGA study suggested that the microcapsules had good heat resistance. The fatty acid composition, the content of sesamin, sesamolin and vitamin E in CPSO were determined before and after microencapsulation. It showed that the microencapsulation process had almost no effect on the fatty acid composition, sesamin and sesamolin, only Vitamin E was slightly lost during the microencapsulation process. The accelerated storage test showed that microencapsulation significantly increased the oxidative stability of CPSO.

Key words: cold-pressed sesame oil (CPSO), microcapsules, complex coacervation, morphology, oxidative stability

1 Introduction

Sesame seeds possess high nutritional value. They are rich in oils and proteins and are mainly used in oil production. Sesame oil contains polyunsaturated fatty acids and functional ingredients such as sesamin and sesamolin that have the effects of reducing blood fat, protecting the liver, preventing aging and fighting cancer. The production processes of sesame oil mainly include the water generation method, leaching method and pressing method. In recent years, green, safe and pollution-free cold-pressed technology has gradually emerged and has been applied to a variety of oils, such as sesame. The cold-pressed oil does not come in contact with chemical reagents, does not use high-temperature roasting or produce harmful compounds such as trans fatty acids, polycyclic aromatic hydrocarbons and polymers. The cold-pressing method does not destroy the original nutrients of the sesame oil, and the obtained CPSO has a light color and scent. In addition, the sesame cake shows a small amount of protein denaturation and a high utilization rate. However, the oxidative stability of CPSO is poor when compared with hot-pressed sesame oil. The conversion of sesamolin to sesamol and Maillard antioxidant products under roasted and pressed at high temperature are the main reasons for the strong oxidative stability of hot-pressed sesame oil. CPSO pressed at a room temperature does not contain sesamol and will not produce Maillard antioxidant products. Therefore, CPSO has poor oxidative stability and a short shelf life, which restricts the use and development of CPSO.

Microcapsule technology is extensive application in the food industry, such as for flavors, minerals, vitamins, oils and bioactive substances. Some functional oils possess a variety of unsaturated fatty acids, such as fish oil, linseed oil, nut oil and olive oil, which resulting in extremely poor oxidative stability, exactly, the application of microcapsule technology on those oil can solve the problem perfectly and there are already many research results. The use of microcapsule technology can not only significantly enhance...
their oxidative stability but also improve their characterization. In addition, microcapsule technology can control the release rate of the core material, cover the unpleasant smell of the core material, and reduce the use and the toxic side effects of core material\textsuperscript{8\textendash}10.

There are many methods to prepare microcapsules. However, complex coacervation is a classical method. Complex coacervation is a combination of two oppositely charged hydrocolloid solutions, causing an interaction and precipitation of the composite polymer. Proteins and polysaccharides are commonly used in the concentrated polymer phase. In particular, gelatin is widely used in combination with gum Arabic as wall materials for complex coacervation. The solubility of the components in the solution decreases and gradually aggregates to form microcapsules when the temperature, pH or concentration of the mixture solution changes. The collection is carried out by filtration or centrifugation and finally lyophilization to prepare a microcapsule powder. Compared with other methods, this method has many advantages. For instance, method is simple in operation and mild in preparation conditions, and the core material can be protected to the utmost extent. In addition, the microcapsules prepared by complex coacervation show high encapsulation efficiency, high yield, strong oxidative stability and slow release\textsuperscript{11\textendash}15.

However, the research of microcapsule technology for CPSO by complex coacervation has rarely been studied. Recently, our lab have already developed the best process parameters for CPSO microcapsules in previous research, so this paper focuses on the characterization and oxidative stability of CPSO microcapsules. The morphology of the CPSO microcapsules was observed by a particle image analyzer, a laser particle size distribution analyzer and scanning electron microscopy (SEM). The characteristics of CPSO microcapsules were analyzed by Fourier transform infrared spectroscopy (FTIR) and a thermogravimetric analysis (TGA). The oxidative stability of CPSO microcapsules was investigated by accelerating the storage test and measuring the peroxide value (PV) of CPSO.

2 Materials and Methods

2.1 Materials

Gelatin (A type) and food grade gum Arabic were provided by Anwen Hydrosol Technology Co. Ltd. (Tianjin, China). CPSO was purchased from Yanzhuang Oil Co. Ltd. (Anhui, China). Food grade transglutaminase was obtained from Dongsheng Food Technology Co. Ltd. (Taixing, China). Food grade glacial acetic acid was obtained from Shengxin Food Ingredients Co. Ltd. (Zhengzhou, China). Sodium hydroxide, petroleum ether (30\textendash}60°C), anhydrous ethanol, hydrochloric acid, and potassium iodide of analytical grade were provided by Tianli Chemical Reagent Co. Ltd. (Tianjin, China). Methanol and n-hexane of chromatographic purity grade were obtained from Komiou Chemical Reagent Co. Ltd. (Tianjin, China).

2.2 Preparation of microcapsules

CPSO microcapsules were prepared by complex coacervation. Transglutaminase was used as the cross-linking agent. The optimal parameters were determined in our previous study as follows\textsuperscript{16}. A gelatin solution (1.0\%, w/v) and a gum Arabic solution (1.0\%, w/v) were dissolved in distilled water to prepare a mixture. The ratio of wall to core was 2:1, and then CPSO was added into the mixture gradually with homogenization with a high-speed dispensing machine (FM200, Shanghai Fluke Company) at 10000 rpm for 2 min to form an emulsion. The magnetic stirring speed was adjusted to 200 rpm for 20 min, and a complex coagulation reaction began to occur. Acetic acid solution (10\%, v/v) was added to adjust the pH of the reaction system to 4.0. The amount of transglutaminase added was 0.125 g/100 mL emulsion in the low temperature curing treatment. The microcapsule suspension was filtered using a sand core funnel and rinsed with distilled water. The produced wet microcapsules were freeze-dried at \textasciitilde}40°C for 24 h in a lyophilizer (LGJ-10C, Beijing Sihuan Scientific Instrument Factory, China).

2.3 Encapsulation Efficiency (EE) of the microcapsules

The surface oil content and total oil content of the microcapsules were determined using the same extraction method described in a previous study\textsuperscript{17}.

\[ \text{EE (\%)} = \frac{\text{total amount of oil} - \text{surface oil}}{\text{initial amount of oil}} \times 100\% \]

2.4 Microscopic morphological analysis

The overall microscopic morphology of the microcapsules was observed using a particle image analyzer (BT-1600, Baite, China). Wet microcapsules were fixed on a glass slide. The cover slips were pressed and the sample was observed under an optical microscope.

2.5 Particle size analysis

The microcapsules were dispersed in distilled water, and their particle sizes were measured using a laser particle size analyzer (BT-9300H, Baite, China).

2.6 Morphology analysis

The morphology of the microcapsules was studied by scanning electron microscopy (S-3400N, Hitachi, Japan). Dry microcapsules were fixed on a table with double-sided tape, and the excess powder was blown off. The microcapsules were coated with gold by a gold sputter coater in a high-vacuum evaporator. The acceleration voltage was 2.5 kV, and the magnification was 300\textendash}1000 times.
2.7 FTIR analysis
Fourier transform infrared spectroscopy (FTIR) analysis was performed with a FTIR instrument (Tensor 27, Bruker, Germany). The dry microcapsules were ground with KBr to form disks at a pressure of 10 tons.

2.8 Thermal analysis
Thermal analysis of the dry microcapsules was performed with a thermal gravimetric analyzer (TGA/DTA-TG 209, Netzsch, Germany) under nitrogen flow, in the temperature range 25-600°C, at a heating rate of 10°C/min, and the temperature was raised from 20°C to 600°C.

2.9 Fatty acid composition analysis
The fatty acid composition of CPSO and encapsulated CPSO (total oil extracted from CPSO microcapsules with the method described in 2.3) were determined using the same extraction and determination methods described in a previous literature.

2.10 Sesamin, sesamolin and vitamin E content of CPSO and encapsulated CPSO analysis
Extraction methods of sesamin and sesamolin were according to the literature. Sesamin and sesamolin were determined by a HPLC (e2695, Waters, USA) equipped with a UV detector (2489, Waters, USA) and a 5 μm Sunfire C18 HPLC column (250 mm × 4.6 mm, Waters, USA). The mobile phase was 70% methanol in water with a rate of 0.8 mL/min and 10 μL was injected. Wavelength absorbance of sesamin and sesamolin was set at 287 nm.

The content of vitamin E of CPSO and encapsulated CPSO was determined using the same extraction and determination methods described in a previous literature.

2.11 Oxidative stability of the microcapsules
An accelerated oxidation experiment was used to determine the oxidative stability of the CPSO microcapsules. Dry microcapsule powder and blank oil were both placed in sealed amber color glass bottles and stored in an incubator at 60°C for 30 days. Suitable microcapsule powders were removed every 3 days to measure the peroxide value (PV). The extraction method of oil from microcapsules was based on a previous research. The PV was characterized according to the AOCs Official Method Cd 8-53.

3 Results and Discussion

3.1 Encapsulation Efficiency (EE) of the microcapsules
The encapsulation efficiency of CPSO microcapsules was determined. The results obtained showed that the total oil content of the CPSO microcapsules was 44.67% and the surface oil content was 4.35%. The encapsulation efficiency of the CPSO microcapsules was 90.25%, which was higher than that of other experimental results. Qv et al. studied lutein microcapsules by complex coacervation and obtained 85.32% encapsulation efficiency. Da Silva et al. found 86.04% encapsulation efficiency for microcapsules containing B. lactis produced by complex coacervation. In addition, Kaushik et al. determined that the encapsulation efficiency of the microcapsules of flaxseed oil by complex coacervation was 87%. Moreover, Rutz et al. studied the microcapsules of palm oil by complex coacervation and showed encapsulation efficiencies ranging from 22.25% to 62.41%. The encapsulation efficiencies of the microcapsules of propolis extract by complex coacervation was 72.01% found by Nori et al.

According to our analysis, the main reason for high encapsulation efficiency in this experiment is the addition of transglutaminase in the process of encapsulation and complex coacervation. Transglutaminase is able to create covalent crosslinks of inter- or intramolecular ε-(γ-glutamine)-lysine isopeptidic bonds through catalysis of the acyl transfer reaction from a γ-carboxyamide group in protein-bound glutamine residues (acyl donor) to an α-amino group in a protein-bound lysine residue (acyl acceptor). This cross-linking reaction is not only conducive to the formation of high molecular weight protein polymer, but also can improve the binding strength and the thermal stability of the cross-linked proteins.

3.2 Microscopic morphological analysis
The microscopic morphology of the wet blank microcapsules and the CPSO microcapsules were observed by optical microscopy as shown in Fig. 1.

Figure 1 (a) shows that the blank microcapsules exhibited a completely transparent spherical structure, with uniform dispersion and a smooth surface. This confirmed the formation of the complex coacervation between gelatin and gum Arabic. As shown in Fig. 1 (b), the wet CPSO microcapsules exhibited a transparent multinuclear structure. The core material was divided into portions, which were encapsulated into the wall material. From the morphology of the microcapsules, it can be seen that smaller-sized microcapsules are aggregated together and encapsulated again by the wall material to form larger multinuclear microcapsules. The morphological properties were in accordance with those obtained.

According to the above research conclusions, we know that the core material was successfully embedded into the microcapsules, which should provide greater protection of the core material. Furthermore, CPSO microcapsules were successfully formed by the complex coacervation method. The microscopic morphological structure of the microencapsulated microcapsules was good and exhibited a transparent multinuclear structure under the optical microscope.
3.3 Particle size and morphology analysis
The particle size of the wet CPSO microcapsules obtained by the laser particle size analyzer is shown in Fig. 2 (a). The cumulative ratio accounted for approximately 75.72% and was mostly distributed between 40 μm and 160 μm. The average particle diameter of the wet CPSO microcapsules was approximately 117.1 μm. In general, the particle sizes of the microcapsules were normally distributed and the range of particle size distribution was relatively narrow, which indicated that the particle consistency of microcapsules was good and the particle size was relatively uniform.

The morphology of the dry CPSO microcapsules were observed by scanning electron microscopy as presented in Fig. 2 (b). The dry CPSO microcapsules were not perfectly spherical in shape, and their surfaces had many dents and wrinkles because the microcapsule walls were directed inward toward the inside of the microcapsules due to the moisture volatilization during freeze drying. Although the surface of the microcapsules had obvious wrinkles, there were no cracks or small pores, and the structure of the microcapsule wall was relatively complete. Xiao et al. also observed the same result. It could be inferred that the microcapsule had a good protective effect on the core material.

3.4 FTIR analysis
The FTIR spectra of the CPSO microcapsules, gum Arabic and gelatin are shown in Fig. 3. Gum Arabic is a polysaccharide containing -OH and -COO- groups, and the aqueous solution was negatively charged. The FTIR spectrum of gum Arabic indicated a strong characteristic hydroxyl peak at 3423 cm⁻¹ and a low intensity characteristic carboxyl group peak at approximately 2850-2929 cm⁻¹. Gelatin is a natural high molecular weight protein with typical amphoteric characteristics, and gelatin is positively charged in acidic solution. The FTIR spectrum of gelatin
suggested a strong characteristic amino peak at 3459 cm\(^{-1}\), the band at 2931 cm\(^{-1}\) was assigned to the C-H telescopic vibration, and the absorption bands at 1633 cm\(^{-1}\), 1537 cm\(^{-1}\) and 1234 cm\(^{-1}\) were assigned to amide I, amide II and amide III, respectively\(^{29,30}\).

The FTIR spectrum of the CPSO microcapsules did not present any new absorption signals except for a small peak shift when compared with the spectra of gum Arabic and gelatin, which indicates that gelatin and gum Arabic formed complex coacervation by the electrostatic interaction between the positive and negative charges in the acidic solution\(^{32}\).

3.5 Thermal analysis

The thermal analysis of the CPSO microcapsules was presented in Fig. 4. The mass loss about 5% recorded before 100°C indicates a small amount of adsorbed water on the microcapsule surface. The mass loss less than 3% recorded from 100°C to 220°C attributed to the bound water in the wall materials of the microcapsules. Rapid thermal degradation of the microcapsules starting from 220°C and finishing at 480°C with a maxim weight loss of approximately 70%. It was supposed that in this stage, gelatin and gum Arabic were oxidized and decomposed, the wall of the CPSO microcapsules were broken and the core materials were released, the CPSO was evaporated, oxidized and degraded. Because of the high stability of the cross-linked proteins of microcapsule wall, the post degradation temperature of the capsule wall coincided with the initial temperature of CPSO oxidative degradation and volatilization, which made it difficult to separate them clearly in the curve in Fig. 4. The temperature varied from 480°C to 600°C, and the weight loss of the microcapsules was slow since the CPSO microcapsules had already been completely carbonized. The thermal analysis showed that the highest heat resistant temperature of the CPSO microcapsules was 220°C. Similar results were also obtained\(^{33,34}\). In short, the CPSO microcapsules had good thermal stability and could effectively protect the core material.

3.6 Effect of microencapsulation on fatty acid composition of CPSO

The percentage content of fatty acid composition is an important indicator for determining the stability and nutritional value of oil. Palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), \(\alpha\)-linolenic acid (18:3) and arachidic acid (20:0) are the main fatty acid components of CPSO. In this study, the main fatty acid composition of CPSO and encapsulated CPSO were determined. From the data in the Table 1, it can be seen that the percentage change of the fatty acid composition in the CPSO is extremely small and can be ignored. It can be seen that the microencapsulation process has almost no effect on the fatty acid composition in CPSO.

3.7 Effect of microencapsulation on the content of sesamin, sesamolin and vitamin E in CPSO

Sesamin, sesamolin and Vitamin E are the unique nutrients in sesame oil. In this study, their contents in CPSO and encapsulated CPSO were measured. From the data in the Table 2, it can be seen that the content of sesamin and sesamolin in CPSO has little change after encapsulated, which means that sesamin and sesamolin are relatively stable and the microencapsulation process has little effect on them. However, the Vitamin E in the CPSO was reduced after encapsulation, which may be caused by the influence
of the acid-base environment during the preparation of microparticles.

### 3.8 Oxidative stability of the microcapsules

The PV reflects the degree of oil oxidation at the initial stage. In the accelerated oxidation experiment, the oxidative stability of blank and encapsulated CPSO was assessed by determining the PV during storage at 60°C for 30 days as shown in Fig. 5.

Initially, the PV of the encapsulated CPSO was higher than that of the blank CPSO. The increase in the PV of the encapsulated CPSO was due to the conditions during the preparation of the microcapsules. Over time, the PV of the blank oil increased rapidly. In contrast, the PV of the encapsulated oil rose very slowly. The Codex Alimentarius Commission has stipulated that the safe upper limit of PV of edible oil is 10 mmol/kg, and the PV of the blank oil after 18 days was close to 10 mmol/kg. In contrast, the PV of the encapsulated CPSO was only 4 mmol/kg after 30 days.

This result was consistent with many similar findings. They indicated the ability of the microcapsules to restrain the development of rancidity and increase the oxidative stability of foods. Therefore, it was confirmed that the encapsulated oil showed an increased oxidative resistance compared with the blank oil, and the microencapsulation significantly improved the oxidative stability of the CPSO. The microencapsulation could effectively prevent the oxidative rancidity of CPSO and prolong its shelf life.

### 4 Conclusion

The present study obtained CPSO microcapsules by complex coacervation and improved the characterization and enhanced the oxidative stability of CPSO. The particle image analyzer, laser particle size distribution analyzer and SEM analysis indicated that the average particle size was approximately 117.1 μm, and many oil droplets were encapsulated by complex coacervation to form a multinuclear spherical microparticle. Although the surface of the dry microcapsules shrunk, there were no cracks. The FTIR study confirmed that the process of complex coacervation was formed between gelatin and gum Arabic by an electrostatic interaction. The TGA study showed that the microcapsules had good heat resistance.

The determination of fatty acid content, sesamin, sesamolin and vitamin E of CPSO before and after microencapsulation suggested that the microencapsulation process had almost no effect on the fatty acid component, sesamin and sesamolin in CPSO, vitamin E was slightly lost during the microencapsulation process. In addition, the accelerated storage test at 60°C for 30 days revealed that the PV of the blank CPSO increased rapidly and was much larger than the microencapsulated CPSO. Finally, the oxidative stability was remarkably enhanced, and the applications of CPSO were expanded by complex coacervation.

### Table 1 Fatty acid composition of CPSO and encapsulated CPSO.

<table>
<thead>
<tr>
<th></th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>20:0</th>
<th>22:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPSO</td>
<td>8.99 ± 0.02</td>
<td>0.13 ± 0.03</td>
<td>5.58 ± 0.01</td>
<td>38.84 ± 0.03</td>
<td>45.23 ± 0.01</td>
<td>0.61 ± 0.01</td>
<td>0.49 ± 0.02</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>encapsulated CPSO</td>
<td>9.03 ± 0.03</td>
<td>0.10 ± 0.01</td>
<td>5.56 ± 0.02</td>
<td>38.86 ± 0.02</td>
<td>45.22 ± 0.01</td>
<td>0.61 ± 0.01</td>
<td>0.51 ± 0.01</td>
<td>0.13 ± 0.01</td>
</tr>
</tbody>
</table>

### Table 2 The content of sesamin, sesamolin and Vitamin E in CPSO and encapsulated CPSO.

<table>
<thead>
<tr>
<th></th>
<th>Sesamin (mg/100 g)</th>
<th>Sesamolin (mg/100 g)</th>
<th>Vitamin E (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPSO</td>
<td>645.89 ± 6.43</td>
<td>379.39 ± 3.68</td>
<td>41.83 ± 0.03</td>
</tr>
<tr>
<td>encapsulated CPSO</td>
<td>637.85 ± 4.19</td>
<td>374.85 ± 3.62</td>
<td>37.41 ± 0.04</td>
</tr>
</tbody>
</table>

Fig. 5 PV of the (a) CPSO and (b) encapsulated CPSO during storage at 60°C for 30 days.
Cold-pressed Sesame Oil Microcapsules

Acknowledgements
We sincerely acknowledge the financial support by the earmarked fund for Modern Agro-industry Technology Research System (CARS14-1-29).

The Authors declare that there is no conflict of interest.

References
24) Nori, M.P.; Favaro-Trindade, C.S.; de Alencar, S.M.;


